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Assessing the effectiveness of Zn-EDTA and Fe-EDTA in agronomic biofortification of barley (*Hordeum vulgare* L.) in alluvial soils of the subtropical region of Uttarakhand

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Abstract
Micronutrient deficiencies also renowned as "Hidden hunger" is a concerning health issue in modern day scenerio. Trace elements like Zinc and Iron are required for normal biochemical and physiological bodily functions of a person. Meager intake of these minerals due to a nutrient deficient diet, triggers various diseases and disorders. Agronomic biofortification is a strategic augmentation of the nutritional profile of primary food crops by using fertilizers. A field experiment was conducted during the rabi season of 2024-25, at Agronomy Research Field of Department of Agronomy, DIBNS Manduwala, Dehradun with the aim of identifying the effectiveness of zinc and iron chelate (EDTA) fertilizers in agronomic biofortification of barley (variety RD 2552). The study was carried out in randomized block design with seven treatments and three replications. The results of the study revealed that T7 (RDF + Zn-EDTA @ 0.75% + Fe-EDTA @ 0.75%) significantly increased the grain zinc (62.41 mg/kg), iron (80.05 mg/kg) and protein content (19.59%) also T7 maximized the biological performance of crop in terms of growth attributes *viz* plant height, tillers number per plant, dry weight/plant, which collectively translated into maximum yield and associated traits.

Keywords: Barley, biofortification, hidden hunger, zinc-EDTA, iron-EDTA, nutitional quality, foliar application

1. Introduction
Barley (*Hordeum vulgare* L.) is one of the first grains to be farmed by human civilization and has been cultivated since prehistoric times in numerous agroclimatic conditions throughout the continents. Archaeological surveys uncovered traces of its domestication around 10 millenia back in past and believed to be orginated from a uncultivated species *Hordeum spontaneum* (Badr *et al*., 2000) [1]. Barley cultivation as a winter-season cereal is widely spread in the alluvial soils of northern regions of India including the hilly regions of Uttarakhand, Himachal Pradesh and Kashmir (Verma *et al*., 2011) [2]. Whole grain barley offers a premium nutrtional profile with dietary fiber content of 14.8 g/100g, iron content of 6 mg/100g, calcium content of 50 mg/100g, zinc content of 3.3 mg/100g (Langridge, 2018) [3].

Micronutrient deficiencies also designated as "hidden hunger" portray a big global health issue in current age. This condition of nutrient deficiency occurs mainly due to insufficient consumption of essential micronutrients owing to the micronutrient deficient diet (Sakellariou & Mylona, 2020) [4]. Anaemia caused by Fe deficiency affects more than 1.2 billion people globally, while the Fe deficiency without clear symptoms of anaemia believed to be even more widespread (Camaschella, 2019) [5]. In womens, during pregnancy occurrence of Zn deficiency poses detrimental health risks to both mother and fetus. It is estimated that globally approx. 82% of gestating women are affected by Zn deficiency (Noor *et al*., 2025) [6].

Biofortification is a technique of elevating the mineral concentration in etable part of primary crops. It offers a long term and sustanable approach in increasing dietary nutrient consumption especially among the underprivileged sections of society which is most affected with the condition of hidden hunger (Saltzman *et al*., 2013) [7]. Nowdays various approaches of biofortifying food crops are available such as genome editting, molecular plant breeding and agronomic interventions like fertilizers application (Cakmak, 2008) [8].

Application of micronutrient fertilizers can enhance the mineral content like Zn and Fe, in consumable parts of foods crops (Prasad & Shivay, 2020) [9].

Therefore, this study aims at assesing the effectiveness of zinc-EDTA and iron-EDTA fertilizers in biofortification of barley.

2. Materials and Methods
2.1 Experimental Site
Geographically, the experiment site is situated at at 30.38° N latitude and 77.94° E longitude, at an elevation of 648m above sea level.

2.1.1 Weather of Experimental Site
During the crop growth period, the maximum monthly rainfall of 36.6 mm was recorded in the month of December and lowest of 7.0 mm monthly rainfall was recorded in january. The highest average monthly temperature of 35.8°C was observed in April, while the lowest was recorded in December (3°C). The average relative humidity peaked in November month (95.6%) and dropped to its minimum in month of April (26.3%).

2.1.2 Soil Characteristics of Experimental Field
The initial soil test results revealed that the soil of study site was slightly acidic with pH of 5.98, rich in organic carbon (1.35%), low electrical conductivity (0.12 dS/m), available nitrogen content was low (268.44 kg/ha), high in phosphorous (99.98 kg/ha), iron (34.38 ppm), zinc (1.34 ppm) and medium levels of potassium (227.3 kg/ha).

2.2 Experimental Details
The experiment was arranged in a Randomized Block Design (RBD) containing seven treatments: T_1 - control (RDF only), T_2 - (RDF + foliar application of Zn-EDTA @ 0.5%), T_3 - (RDF + foliar application of Fe-EDTA @ 0.75%), T_4 - (RDF + foliar application of Fe-EDTA @ 0.5%), T_5 - (RDF + foliar application of Zn-EDTA @ 0.75%), T_6 - (RDF + foliar application of Zn-EDTA @ 0.5% + Fe-EDTA @ 0.5%), and T_7 - (RDF + foliar application of Zn-EDTA @ 0.75% + Fe-EDTA @ 0.75%). Each treatment was replicated three times.
Barley (cv. RD 2552) was sown on 14 November 2024 at spacing of 22cm row to row and 5cm plant to plant. Seed rate of 100 kg/ha was used in the experiment. The crop received 60 kg/ha Nitrogen, 30 kg/ha Phosphorus and 20 kg/ha Potassium supplied via Urea, DAP, MOP respectively. One third dose of Urea was applied as basal dose with full quantity of DAP and MOP and remaining Urea doses were supplied through foliar sprays at tillering and grain filling stage. Zn and Fe were supplied via Zn-EDTA and Fe-EDTA fertilizers. Spray mixture was prepared in 500 litres/ha of water. The foliar applications were implemented at tillering, booting and grain filling stage according to the treatments assigned to experimental plots. After harvesting, grain samples were collected, processed, labelled and further analysis was conducted for estimation of protein, zinc and iron content.

2.4 Statistical Analysis
The collected data from the study was then statistically anayzed using ANOVA for RBD and the critical difference (CD) at the 5% level of significance was applied using the method outlined by Gomez and Gomez (1984) [10].

3. Experimental Results
3.1 Effect of Zn EDTA and Fe EDTA application on growth attributes of barley
Barley growth attributing characters such as plant height, tillers number/plant and dry matter accumulation per plant, showed highly positive response to zinc-EDTA and iron-EDTA foliar feeding (Table 1). The results indicated that, the highest values of growth attributes were recorded under T7 (RDF + foliar application of Zn-EDTA @ 0.75% + Fe-EDTA @ 0.75%). This enhancement in growth parameters could be linked to synergistic effects of Zn and Fe in boosting, chlorophyll formation, enzymes activity and photosynthesis efficiency, which in turn fostered better vegetative growth. Zn has a crucial function in auxin synthesis and protein metabolism, while Fe functions in chlorophyll biosynthesis and electron transport.
Hence their combined effect boosted the cellular functions and overall plant vigor. Similar growth-enhancing effects of zinc and iron application have been reported by Tiwari *et al*. (2023) [11] in barley, Hashim *et al*. (2022) [12] in wheat, Krishnaraj *et al*. (2020) [13] in maize, Sudhagar *et al*. (2019) [14] in rice, and Ramasahayam *et al*. (2020) [15] in pearl millet.

3.2 Effect of Zn-EDTA and Fe-EDTA application on yield attributes and yield of barley
The data presented in table 2, indicates that T7 attained the highest values of yield attributes *viz* productive tillers/plant, spike length, grains/spike and test weight. The highest grain, straw and biological yield were observed under T7 (RDF + foliar application of Zn-EDTA @ 0.75% + Fe-EDTA @ 0.75%).
These improvements are likely due to mutual effects of Zn and Fe in enhancing enzymatic activities, photosynthesis efficiency, nutreint uptake & translocation. This comprehensive improvement in physiology of plant contributed to better vegetative growth, grain development and increased the photosynthate translocation towards reproductive parts, which ultimately boosted the yield parameters and productivity. The present findings align with the results of Tiwari *et al*. (2023) [11] and Babaeian *et al*. (2012) [16], who also observed notable increment in yield and yield attributes of barley following zinc and iron application. Similar benefits were reported in wheat by Hashim *et al*. (2022) [12] and in pearl millet by Shete (2020) [17].

Table 1: Effect of Zn EDTA and Fe EDTA application on growth attributes of barley

Treatments	Plant height(cm)				Number of tillers/plant				dry weight/plant (g)			
	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest
T1	28.18	72.07	91.31	92.72	3.03	3.33	4.67	4.00	0.35	2.81	13.53	15.55
T2	34.54	86.35	106.41	108.96	3.72	4.00	5.00	5.00	0.52	4.85	21.43	25.44
T3	35.10	87.08	104.01	106.54	4.23	5.00	7.67	7.67	0.76	6.38	25.74	31.57
T4	33.05	83.10	100.56	103.06	3.65	4.33	6.67	7.00	0.59	4.84	20.22	24.35
T5	37.79	91.56	111.24	113.47	3.73	5.00	8.33	5.67	0.86	8.25	27.58	34.12
T6	40.66	97.21	115.14	116.58	4.47	6.67	9.67	9.00	1.00	10.13	31.41	39.57
T7	43.78	100.87	119.58	121.11	5.84	7.73	11.00	10.00	1.11	11.16	34.32	43.72
S.Em (±)	0.23	0.40	0.51	0.34	0.07	0.49	0.44	0.67	0.02	0.04	0.13	0.18
C.D (p=0.05)	0.68	1.17	1.49	1.01	0.20	1.44	1.31	1.40	0.05	0.11	0.39	0.52

Table 2: Effect of Zn EDTA and Fe EDTA application on yield attributes and yield of barley

Treatments	Number of productive tillers/plant	Spike length(cm)	Number of grains/spike	Test weight (g)	Biological yield (q/ha)	Grain yield (q/ha)	Straw yield (q/ha)	Harvest index (%)
T1	4.03	8.00	31.42	37.31	68.67	27.35	47.35	40.53
T2	4.30	9.33	34.36	38.68	80.71	33.58	54.08	42.25
T3	4.57	10.00	35.73	40.31	88.60	37.20	58.20	42.74
T4	4.84	9.47	33.64	39.29	84.03	35.39	55.84	42.51
T5	5.10	10.93	37.33	41.51	99.70	42.39	64.89	43.08
T6	5.35	12.67	38.33	42.98	107.19	46.11	68.68	43.46
T7	5.58	15.73	40.08	43.96	108.65	47.48	69.91	44.24
S.Em (±)	0.06	0.53	0.85	0.46	1.25	0.65	0.60	0.36
C.D (p=0.05)	0.18	1.56	2.50	1.35	3.68	1.91	1.78	1.07

3.3 Effect of Zn-EDTA and Fe-EDTA application on quality parameters of barley

Foliar feeding with Zinc-EDTA and Iron-EDTA markedly elevated the grain nutrient density of barley. Post harvest grain analysis showed a elevation in grain Zn, Fe and protein concentrations (Table 3). In comparission to all the treatments, T7 which involved combined application of Zn-EDTA and Fe-EDTA at 0.75% concentration with recommended doses of fertilizers recorded the highest grain protein content of 19.59%, grain Zn concentration of 62.41 mg/kg and grain Fe content of 80.05 mg/kg. The elevation in grain protein concentrations can be attributed to the crucial role of zinc and iron in plant physiology and metabolic activities- Zn promotes the expression of MdNRT1.1 and MdNRT2.4 which are nitrate transporter genes, contributing to better improved uptake of NO_3 (nitrate) from roots. Zn is also required for enzyme activation such as NR, NiR,GS, GOGAT which play very important key role in nitrogen assimilation and protein biosynthesis meanwhile Fe functions in chlorophyll formation and electron transport during photosynthesis, providing the energy required by the enzymes working in protein biosynthesis.

The increment in grain Fe and Zn concentration under T7 (RDF + foliar application of Zn-EDTA @ 0.75% + Fe-EDTA @ 0.75%) can be attributed to the method and timing of application. Foliar feeding bypasses soil-related limitations, which supports the direct absorption of these nutrients into the active tissues. The timing of application at tillering, booting, and grain filling-aligned with key physiological stages of barley, when its nutrient demand is at peak, supporting better growth and development. This might have facilitated better translocation of zinc and iron into the developing grains. Similar outcomes were reported by Ram *et al*. (2024) [18] in wheat and Hadole *et al*. (2024) [19] in summer groundnut, Dhaliwal *et al*. (2021) [20, 21] in indian mustard and chickpea and Saleem *et al*. (2016) [22] in maize, where foliar Zn and Fe sprays significantly enhanced grain nutrient content.

Table 3: Effect of Zn EDTA and Fe EDTA application on grain nutritional quality of barley

Treatments		Protein content (%)	Zinc content (mg/kg)	Iron content (mg/kg)
T1	CONTROL	9.1	30.28	63.02
T2	RDF + Foliar application of Zn EDTA @ 0.5%	10.15	37.48	63.17
T3	RDF + Foliar application of Fe EDTA @ 0.75%	9.80	30.49	73.22
T4	RDF + Foliar application of Fe EDTA @ 0.5%	9.20	30.64	67.01
T5	RDF + Foliar application of Zn EDTA @ 0.75%	11.82	46.36	63.28
T6	RDF + Foliar application of Zn EDTA @ 0.5% + Fe EDTA @ 0.5%	14.46	49.18	74.13
T7	RDF+ Foliar application of Zn EDTA @ 0.75% + Fe EDTA @ 0.75%	19.59	62.41	80.05
S.Em (±)		0.39	0.51	0.65
C.D (p=0.05)		1.16	1.50	1.93

4. Conclusion

The present study shows that combined application of Zn-EDTA and Fe-EDTA at 0.75%, greatly improved the growth dynamics, yield performance, and nutritional quality of barley when treated during the tillering, booting, and grain filling stages. This demonstrates that the use of micronutrient chelate fertilizers can be considered as an effective approach in performing biofortification teachnique to enhance grain nutritional quality, thereby contributing to food and nutritional security. Future research work may focus on optimizing concentrations across various agro-climatic conditions and identifying the long term effect of agronomic biofortification strategy on soil and human health.

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